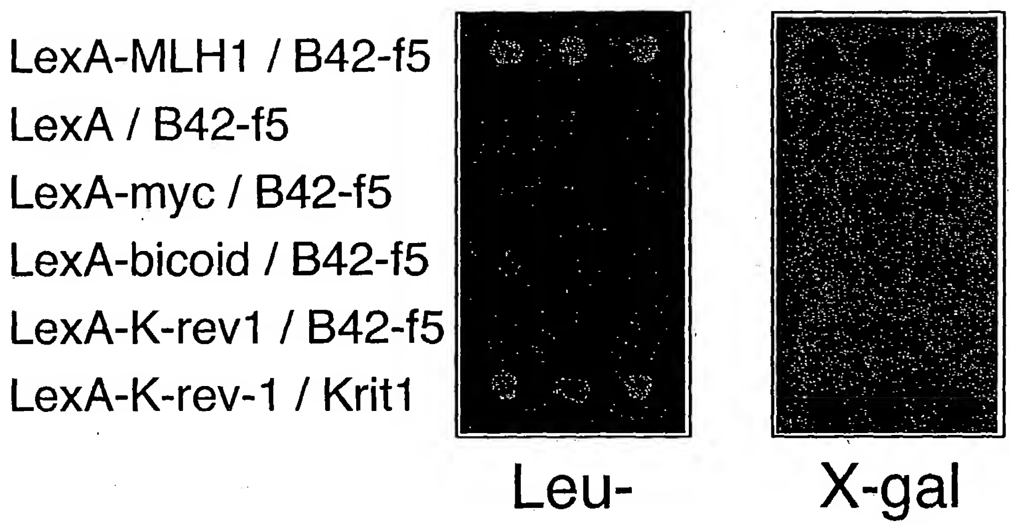

LexA-MLH1 / B42-f5
LexA / B42-f5
LexA-myc / B42-f5
LexA-bicoid / B42-f5
LexA-K-rev1 / B42-f5
LexA-K-rev-1 / Krit1
Leu-
X-gal

Fig. 1

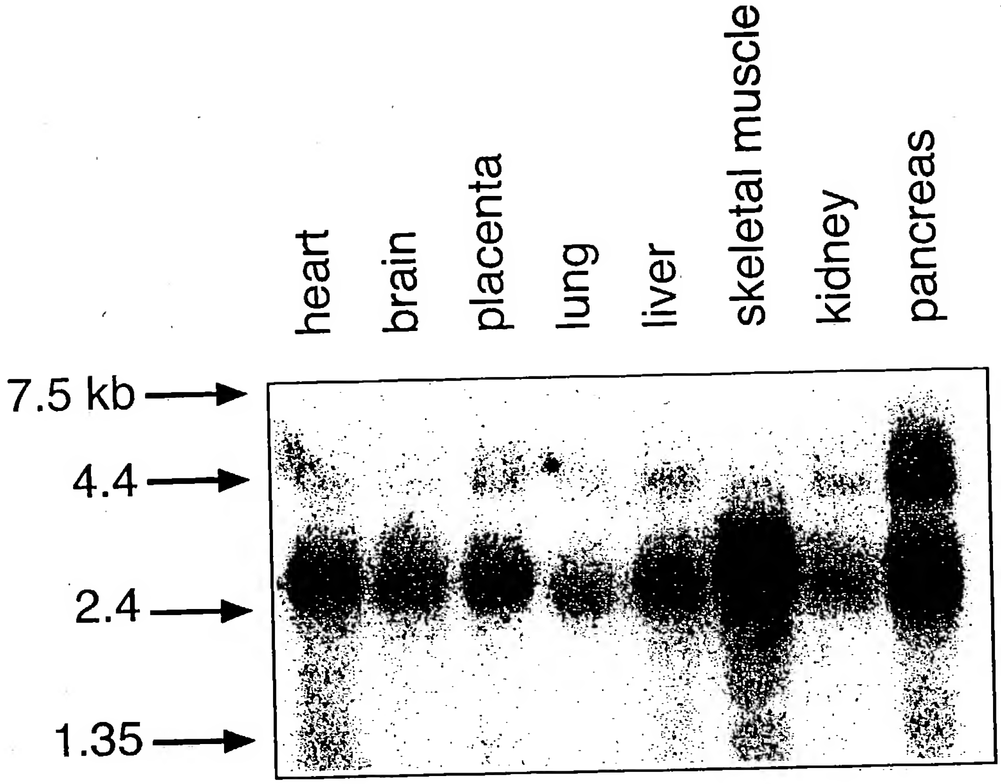

heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
7.5 kb
4.4
2.4
1.35

Fig. 2

```
   1 GGCGGCGTCTGGGGCGCTTTCGCAACATTCAGACCTCGGTTGCAGCCCGGTGCCGTGAGCTGAAGAGGTTTCACATCTTACTCCGCCCCA   90
  91 CACCCTGGGCGTTGCGGCGCTGGGCTCGTTGCTGCAGCCGGACCCTGCTCGATGGGCACGACTGGGCTGGAGAGTCTGAGTCTGGGGGAC  180
                                                        M  G  T  T  G  L  E  S  L  S  L  G  D

 181 CGCGGAGCTGCCCCCACCGTCACCTCTAGTGAGCGCCTAGTCCCAGACCCGCCGAATGACCTCCGCAAAGAAGATGTTGCTATGGAATTG  270
     R  G  A  A  P  T  V  T  S  S  E  R  L  V  P  D  P  P  N  D  L  R  K  E  D  V  A  M  E  L

 271 GAAAGAGTGGGAGAAGATGAGGAACAAATGATGATAAAAAGAAGCAGTGAATGTAATCCCTTGCTACAAGAACCCATCGCTTCTGCTCAG  360
     E  R  V  G  E  D  E  E  Q  M  M  I  K  R  S  S  E  C  N  P  L  L  Q  E  P  I  A  S  A  Q

 361 TTTGGTGCTACTGCAGGAACAGAATGCCGTAAGTCTGTCCCATGTGGATGGGAAAGAGTTGTGAAGCAAAGGTTATTTGGGAAGACAGCA  450
     F  G  A  T  A  G  T  E  C  R  K  S  V  P  C  G  W  E  R  V  V  K  Q  R  L  F  G  K  T  A

 451 GGAAGATTTGATGTGTACTTTATCAGCCCACAAGGACTGAAGTTCAGATCCAAAAGTTCACTTGCTAATTATCTTCACAAAAATGGAGAG  540
     G  R  F  D  V  Y  F  I  S  P  Q  G  L  K  F  R  S  K  S  S  L  A  N  Y  L  H  K  N  G  E

 541 ACTTCTCTTAAGCCAGAAGATTTTGATTTTACTGTACTTTCTAAAAGGGGTATCAAGTCAAGATATAAAGACTGCAGCATGGCAGCCCTG  630
     T  S  L  K  P  E  D  F  D  F  T  V  L  S  K  R  G  I  K  S  R  Y  K  D  C  S  M  A  A  L

 631 ACATCCCATCTACAAAACCAAAGTAACAATTCAAACTGGAACCTCAGGACCCGAAGCAAGTGCAAAAAGGATGTGTTTATGCCGCCAAGT  720
     T  S  H  L  Q  N  Q  S  N  N  S  N  W  N  L  R  T  R  S  K  C  K  K  D  V  F  M  P  P  S

 721 AGTAGTTCAGAGTTGCAGGAGAGCAGAGGACTCTCTAACTTTACTTCCACTCATTTGCTTTTGAAAGAAGATGAGGGTGTTGATGATGTT  810
     S  S  S  E  L  Q  E  S  R  G  L  S  N  F  T  S  T  H  L  L  L  K  E  D  E  G  V  D  D  V

 811 AACTTCAGAAAGGTTAGAAAGCCCAAAGGAAAGGTGACTATTTTGAAAGGAATCCCAATTAAGAAAACTAAAAAAGGATGTAGGAAGAGC  900
     N  F  R  K  V  R  K  P  K  G  K  V  T  I  L  K  G  I  P  I  K  K  T  K  K  G  C  R  K  S

 901 TGTTCAGGTTTTGTTCAAAGTGATAGCAAAAGAGAATCTGTGTGTAATAAAGCAGATGCTGAAAGTGAACCTGTTGCACAAAAAAGTCAG  990
     C  S  G  F  V  Q  S  D  S  K  R  E  S  V  C  N  K  A  D  A  E  S  E  P  V  A  Q  K  S  Q

 991 CTTGATAGAACTGTCTGCATTTCTGATGCTGGAGCATGTGGTGAGACCCTCAGTGTGACCAGTGAAGAAAACAGCCTTGTAAAAAAAAAA 1080
     L  D  R  T  V  C  I  S  D  A  G  A  C  G  E  T  L  S  V  T  S  E  E  N  S  L  V  K  K  K

1081 GAAAGATCATTGAGTTCAGGATCAAATTTTTGTTCTGAACAAAAAACTTCTGGCATCATAAACAAATTTTGTTCAGCCAAAGACTCAGAA 1170
     E  R  S  L  S  S  G  S  N  F  C  S  E  Q  K  T  S  G  I  I  N  K  F  C  S  A  K  D  S  E

1171 CACAACGAGAAGTATGAGGATACCTTTTTAGAATCTGAAGAAATCGGAACAAAAGTAGAAGTTGTGGAAAGGAAAGAACATTTGCATACT 1260
     H  N  E  K  Y  E  D  T  F  L  E  S  E  E  I  G  T  K  V  E  V  V  E  R  K  E  H  L  H  T

1261 GACATTTTAAAACGTGGCTCTGAAATGGACAACAACTGCTCACCAACCAGGAAAGACTTCACTGGTGAGAAAATATTTCAAGAAGATACC 1350
     D  I  L  K  R  G  S  E  M  D  N  N  C  S  P  T  R  K  D  F  T  G  E  K  I  F  Q  E  D  T

1351 ATCCCACGAACACAGATAGAAAGAAGGAAAACAAGCCTGTATTTTTCCAGCAAATATAACAAAGAAGCTCTTAGCCCCCCACGACGTAAA 1440
     I  P  R  T  Q  I  E  R  R  K  T  S  L  Y  F  S  S  K  Y  N  K  E  A  L  S  P  P  R  R  K

1441 GCCTTTAAGAAATGGACACCTCCTCGGTCACCTTTTAATCTCGTTCAAGAAACACTTTTTCATGATCCATGGAAGCTTCTCATCGCTACT 1530
     A  F  K  K  W  T  P  P  R  S  P  F  N  L  V  Q  E  T  L  F  H  D  P  W  K  L  L  I  A  T

1531 ATATTTCTCAATCGGACCTCAGGCAAAATGGCAATACCTGTGCTTTGGAAGTTTCTGGAGAAGTATCCTTCAGCTGAGGTAGCAAGAACC 1620
     I  F  L  N  R  T  S  G  K  M  A  I  P  V  L  W  K  F  L  E  K  Y  P  S  A  E  V  A  R  T

1621 GCAGACTGGAGAGATGTGTCAGAACTTCTTAAACCTCTTGGTCTCTACGATCTTCGGGCAAAAACCATTGTCAAGTTCTCAGATGAATAC 1710
     A  D  W  R  D  V  S  E  L  L  K  P  L  G  L  Y  D  L  R  A  K  T  I  V  K  F  S  D  E  Y

1711 CTGACAAAGCAGTGGAAGTATCCAATTGAGCTTCATGGGATTGGTAAATATGGCAACGACTCTTACCGAATTTTTTGTGTCAATGAGTGG 1800
     L  T  K  Q  W  K  Y  P  I  E  L  H  G  I  G  K  Y  G  N  D  S  Y  R  I  F  C  V  N  E  W

1801 AAGCAGGTGCACCCTGAAGACCACAAATTAAATAAATATCATGACTGGCTTTGGGAAAATCATGAAAAATTAAGTTTATCTTAAACTCTG 1890
     K  Q  V  H  P  E  D  H  K  L  N  K  Y  H  D  W  L  W  E  N  H  E  K  L  S  L  S  *

1891 CAGCTTTCAAGCTCATCTGTTATGCATAGCTTTGCACTTCAAAAAAGCTTAATTAAGTACAACCAACCACCTTTCCAGCCATAGAGATTT 1980
1981 TAATTAGCCCAACTAGAAGCCTAGTGTGTGTGCTTTCTTAATGTGTGTGCCAATGGTGGATCTTTGCTACTGAATGTGTTTGAACATGTT 2070
2071 TTGAGATTTTTTTAAAATAAATTATTATTTGACAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2152
```

Fig. 3

```
hmed1   KEDVAMELER V.........  GEDEEQMMIK RSSECNPLLQ EPIAS
rmecp2  KEDKEGKHEP LQPSAHHSAE  PAEAGKAETS ESSGSAPAVP EASAS

hmed1   AQ.....FGA TAGTECRKSV  PCGWERVVKQ RLFGKTAGRF DVYFI
rmecp2  PKQRRSIIRD RGPMYDDPTL  PEGWTRKLKQ RKSGRSAGKY DVYLI

hmed1   SPQGLKFRSK SSLANYLHKN  GETSLKPEDF DFTVLSKRGI KSR K
rmecp2  NPQGKAFRSK VELIAYFEKV  GDTSLDPNDF DFTVTG.RGS PSR .

hmed1   EDKEGKHEP
rmecp2  .........
```

Fig. 4A

```
       a-hmed1  DPWKLLIATI FLNRTSGKMA IPVLWKFLEK YPSAEVARTA DWRDVSELLK PLGLYDLRAK
  b-endo3ecoli  SPFELLIAVL LSAQATDVSV NKATAKLYPV ANTPAAMLEL GVEGVKTYIK TIGLYNSKAE
      c-gtmrmt  DPYVILITEI LLRRTTAGHV KKIYDKFFVK YKCFEDILKT PKSEEAKDIK EIGLSNQRAE
    d-uvendoml  TPFELLVATV LSAQTTDVRV NAATPAEFAR FPDAHAMAAA TEPELQELVR STGGYRNKAS
   e-mutyecoli  TPYKVWLSEV MLQQTQVATV IPYFERFMAR FPTVTDLANA PLDEVLHLWT GLGY.YARAR

       a-hmed1  TIVKFSDEYL TKQ....... .WKYPIELHG EGKYGNDSY. .......... ...RIFCVNE
  b-endo3ecoli  NIIKTCRILL EQHNGEVPED RAALEA.LPG VGRKTANVVL NTAFGWPTIA VDTHIFRVCN
      c-gtmrmt  QLKELARVVI NDYGGRVPRN RKAILD.LPG VGKYTCAAVM CLAFGKKAAM VDANFVRVIN
    d-uvendoml  AILRLSQELV GRHDGEVPAR LEDLVA.LPG VGRKTAFVVL GNAFGQPGIT VDTHFGRLAR
   e-mutyecoli  NLHKAAQQVA TLHGGKFPET FEEVAA.LPG VGRSTAGAIL SLSLGKHFPI LDGNVKRVLA

       a-hmed1  WKQVHPEDHK LNKYHDWLWE NHEKLSLS.. .......... .......... ..........
  b-endo3ecoli  RTQFAPGKNV EQVEEKLLKV VPA..EFKVD CHHW...LIL HGRYTCIARK PRCGSCIIED
      c-gtmrmt  RYFG.GSYEN LNYNHKALWE LAETLVPGGK CRDFNLGLMD FSAIICAPRK PKCEKCGMSK
    d-uvendoml  RLGFTDETDP GKGRARRGEP VPPARDWTML S.HR...LIF HGRRVCHARR PACGRCPIAR
   e-mutyecoli  RCYAVSGWPG KKEVENKLWS LSEQVTPAVG VERFNQAMMD LGAMICTRSK PKCSLCPLQN

       a-hmed1  ..........
  b-endo3ecoli  LCEYKEKVDI
      c-gtmrmt  LCSYYEKCST
    d-uvendoml  WCPSYAAGET
   e-mutyecoli  GCIAAANNSW
```

Fig. 4B

```
MED1 IASAQFGATA GTECRKSVPC GWERVVKQRL FGKTAGRFDV YFISPQGLKF RSKSSLANYL 60
PCM1 MAEDWLDCPA .......LGP GWKRREVFRK SGATCGRSDT YYQSPTGDRI RSKVELTRYL 53

MED1 HKNGETSLKP EDFDFTVLSK RGIKS 85
PCM1 GPACDLT..L FDFKQGILCY PAPKA 76
```

Fig. 4C

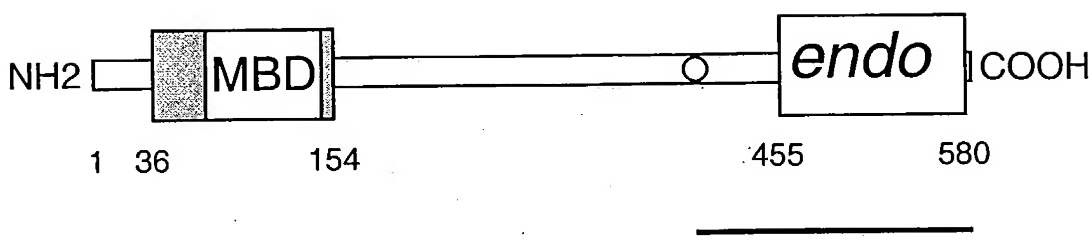


Fig. 5

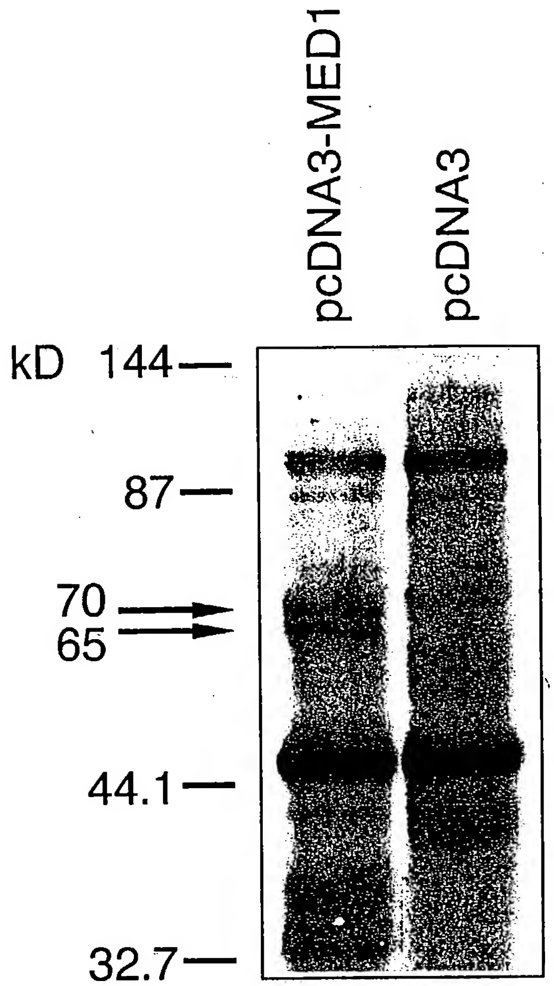
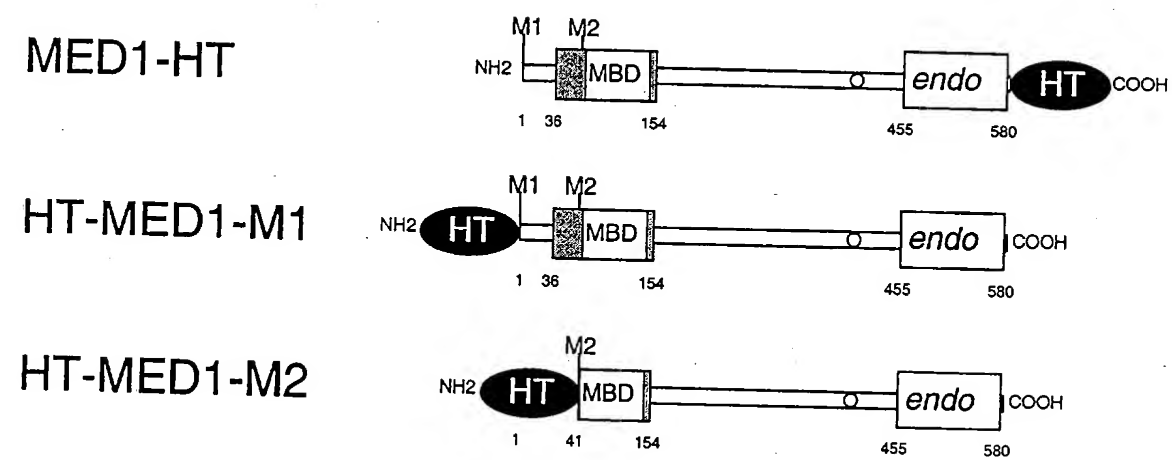


Fig. 6

Fig. 7A


MED1-HT
HT-MED1-M1
HT-MED1-M2
M1
M2
NH2
MBD
endo
HT
COOH
1
36
41
154
455
580

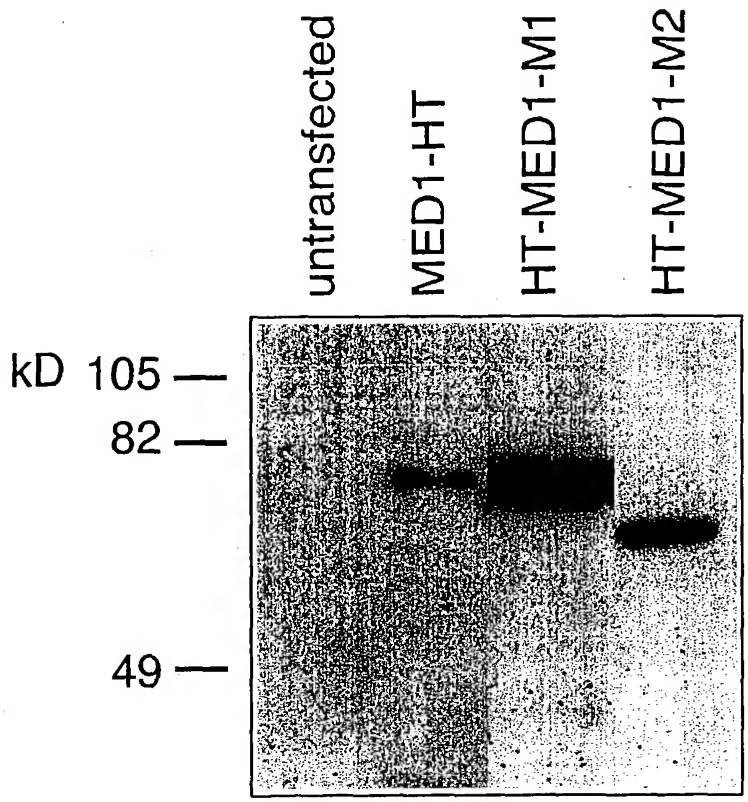


Fig. 7B

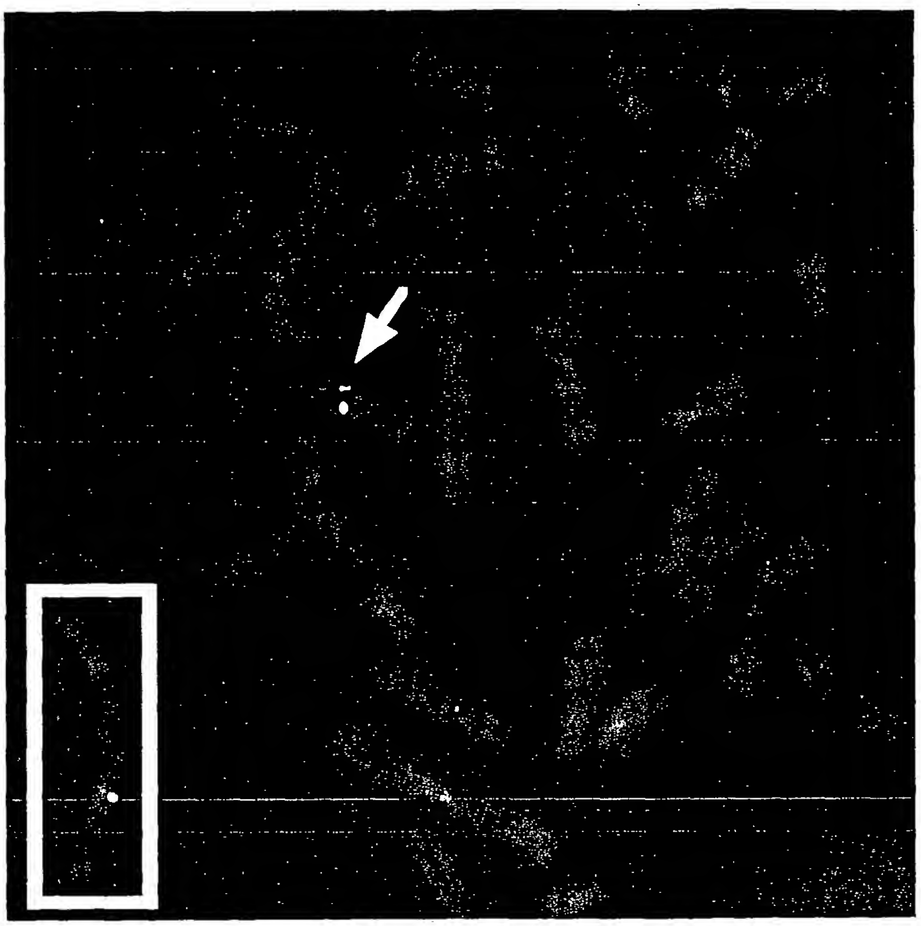


Fig. 8

Fig. 9A

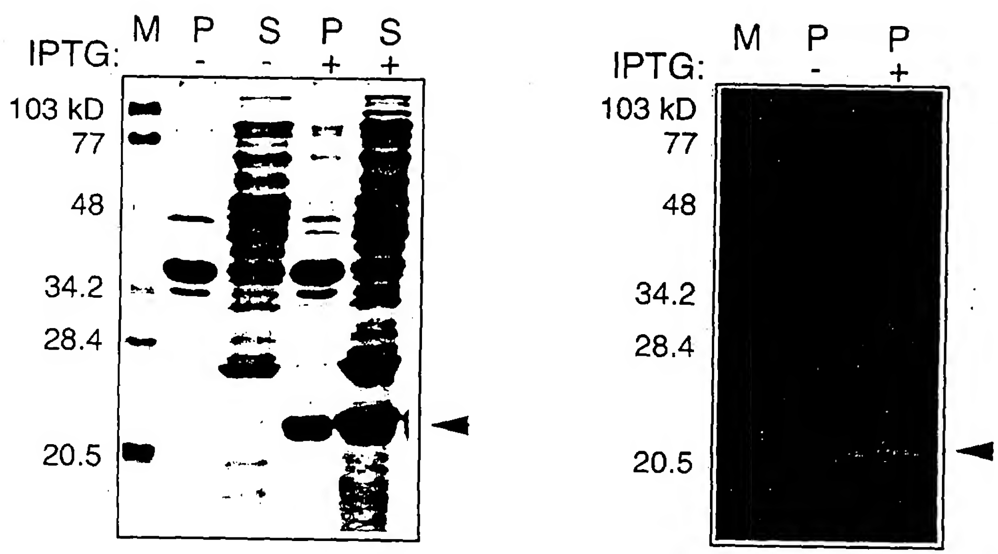
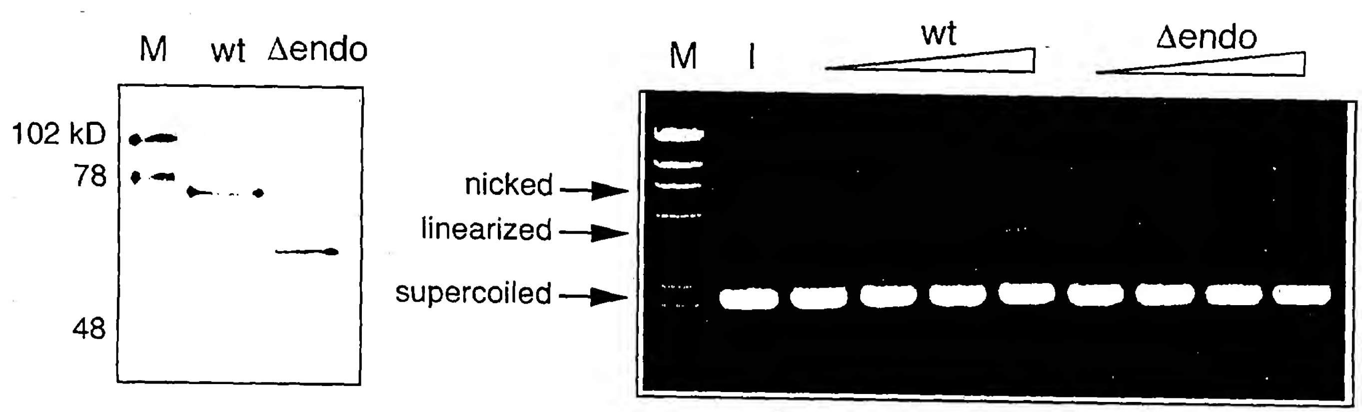

M P S P S
IPTG: - - + +
103 kD
77
48
34.2
28.4
20.5
M P P
IPTG: - +
103 kD
77
48
34.2
28.4
20.5

Fig. 9B


M wt Δendo
102 kD
78
48
M I wt Δendo
nicked
linearized
supercoiled

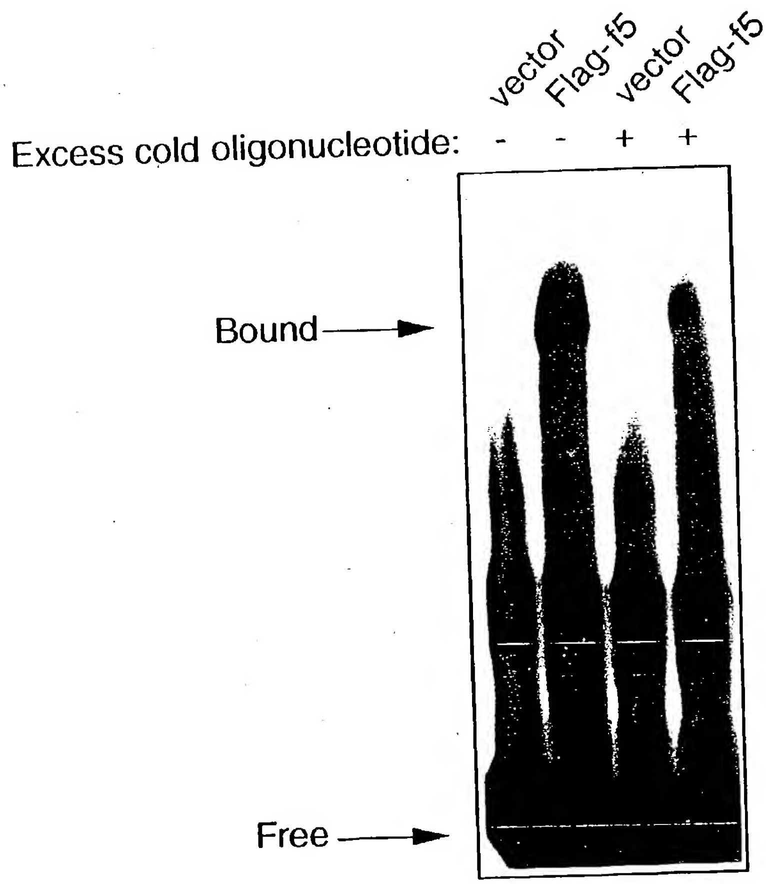

vector
Flag-f5
vector
Flag-f5
Excess cold oligonucleotide: - - + +
Bound
Free

Fig. 10A

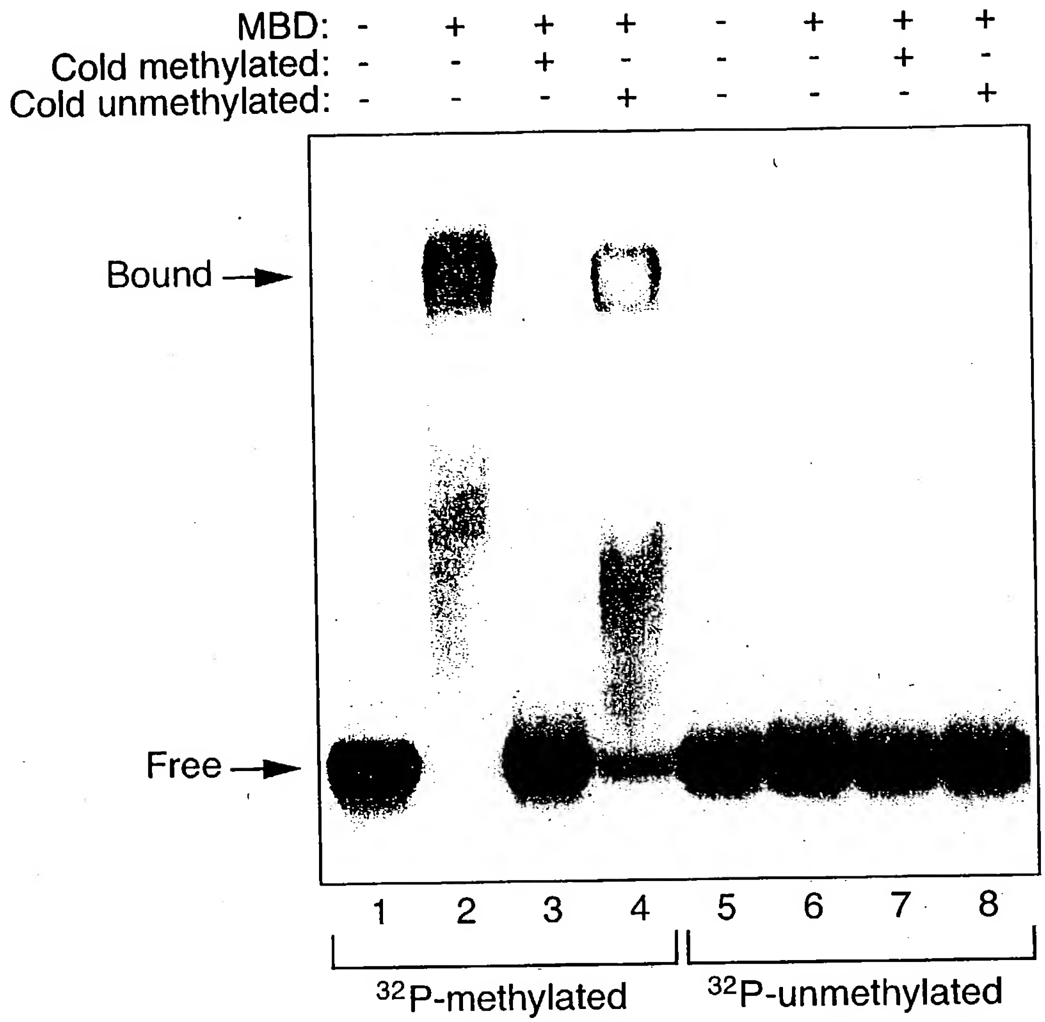

MBD: - + + + - + + +
Cold methylated: - - + - - - + -
Cold unmethylated: - - - + - - - +
Bound
Free
1 2 3 4 5 6 7 8
32P-methylated
32P-unmethylated

Fig. 10B

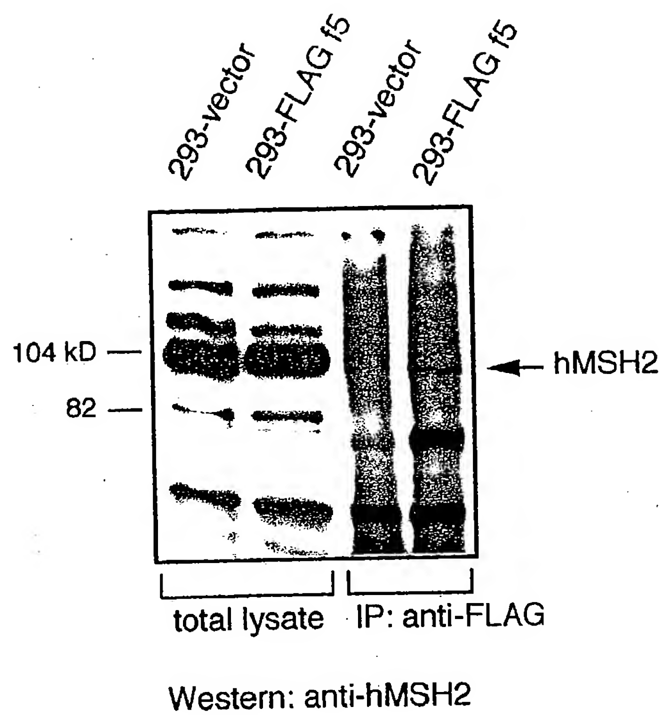

293-vector
293-FLAG f5
293-vector
293-FLAG f5
104 kD
82
hMSH2
total lysate
IP: anti-FLAG
Western: anti-hMSH2

Fig. 11A

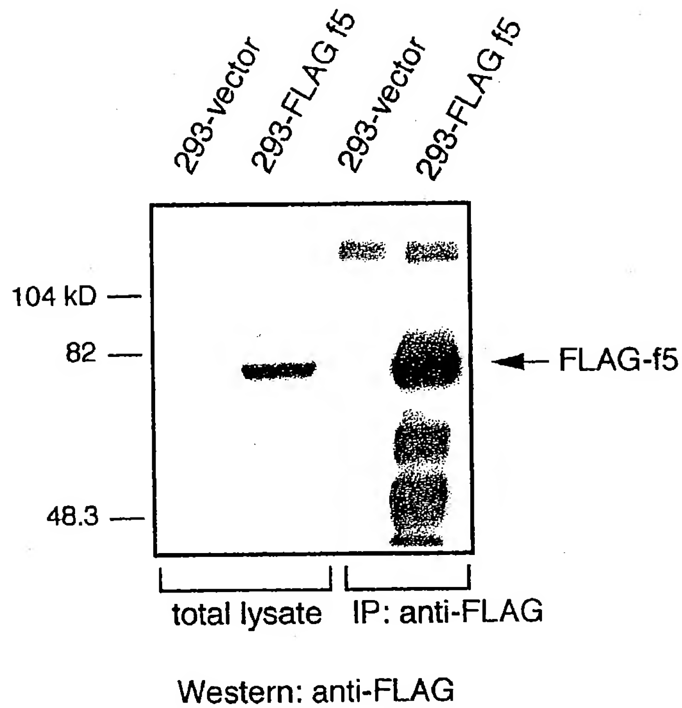
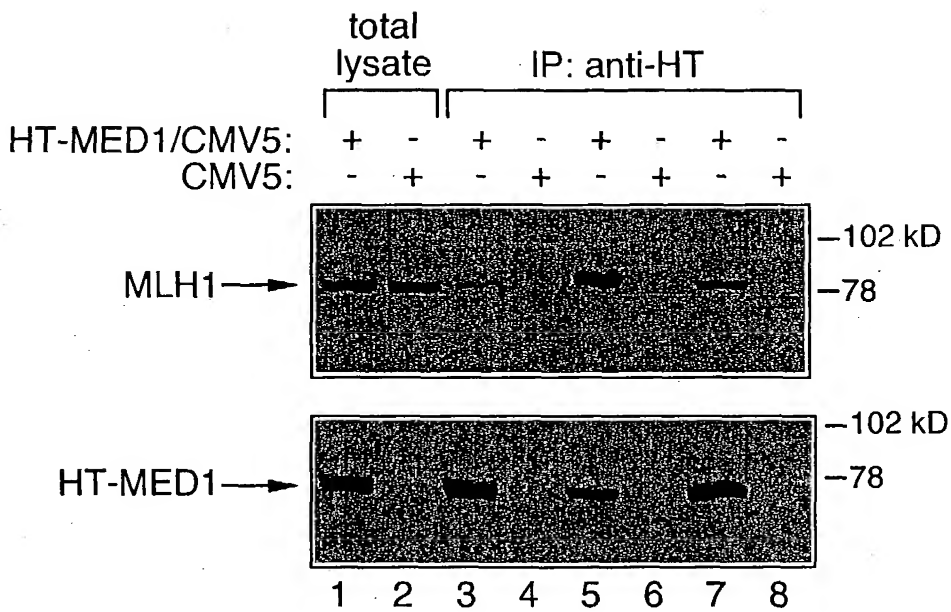

293-vector
293-FLAG f5
293-vector
293-FLAG f5
104 kD
82
48.3
FLAG-f5
total lysate
IP: anti-FLAG
Western: anti-FLAG

Fig. 11B

Fig. 11C



total lysate
IP: anti-HT
HT-MED1/CMV5: + - + - + - + -
CMV5: - + - + - + - +
MLH1
—102 kD
—78
HT-MED1
—102 kD
—78
1 2 3 4 5 6 7 8

Fig. 12A

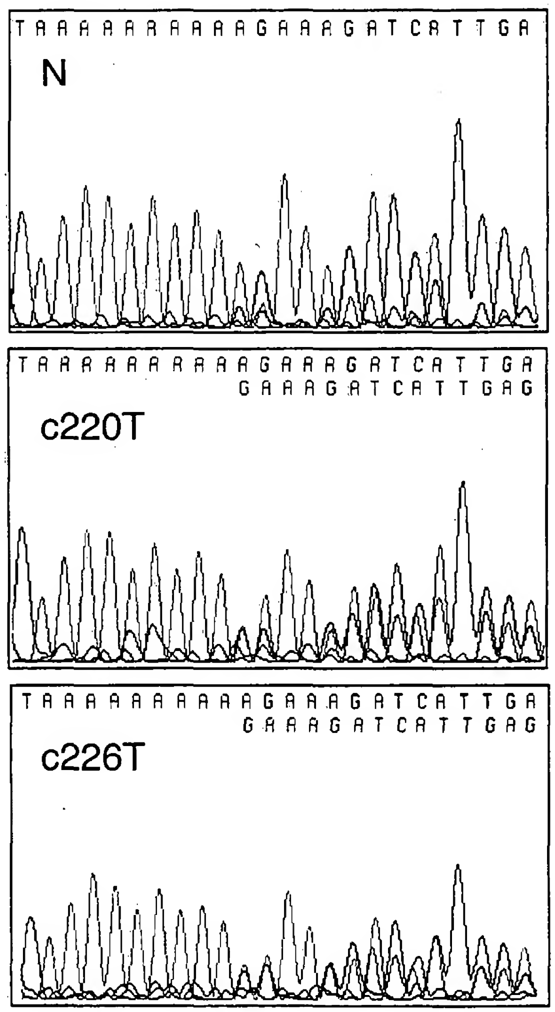


Fig. 12B

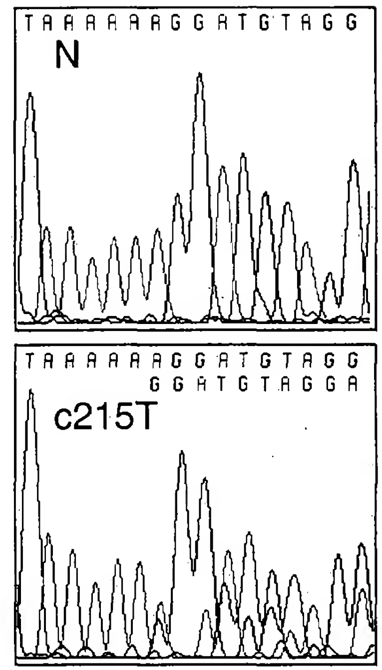
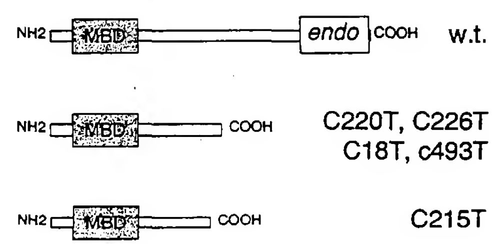


Fig. 12C



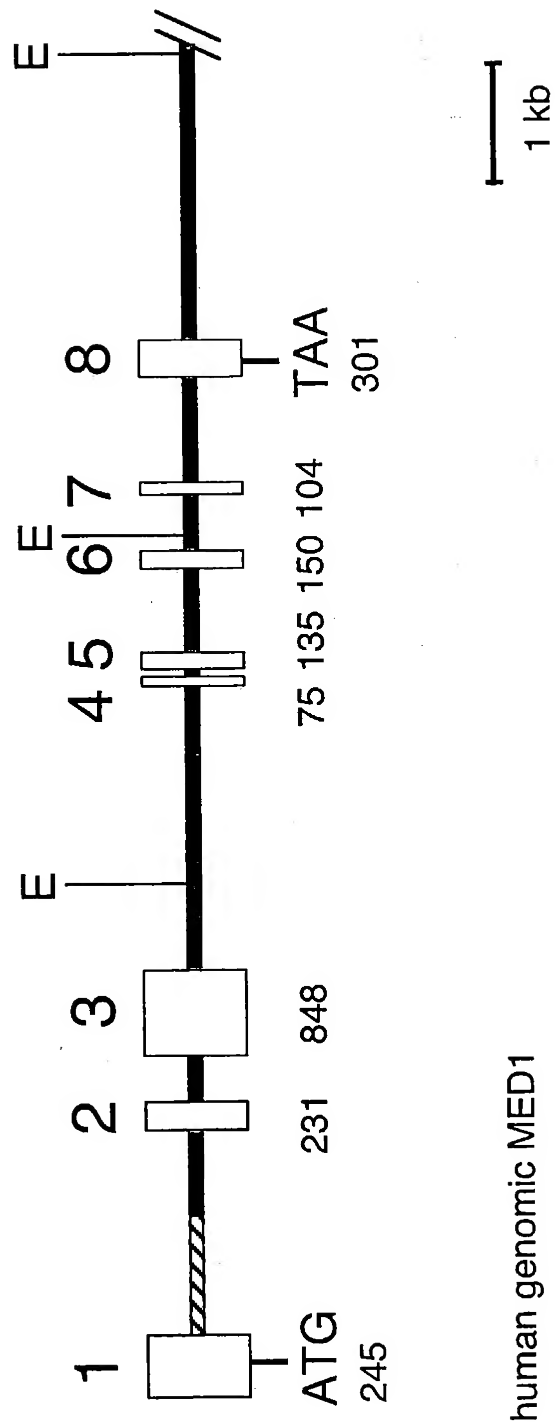

E
E
E
1
2
3
4 5
6
7
8
ATG
245
231
848
75 135 150 104
TAA
301
1 kb
human genomic MED1

Fig. 13

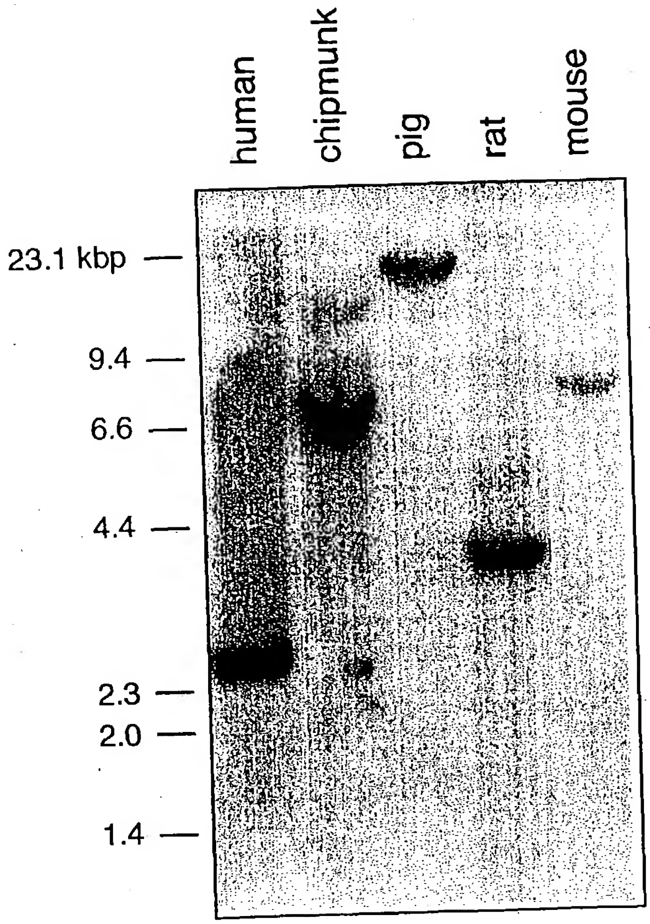


Fig. 14A

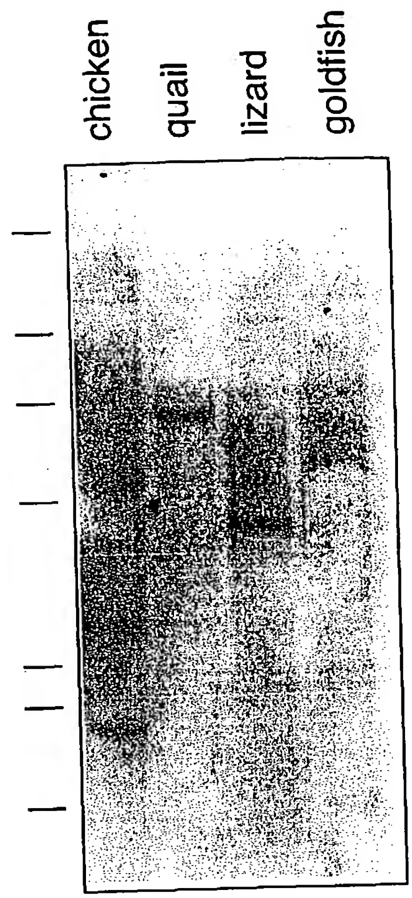
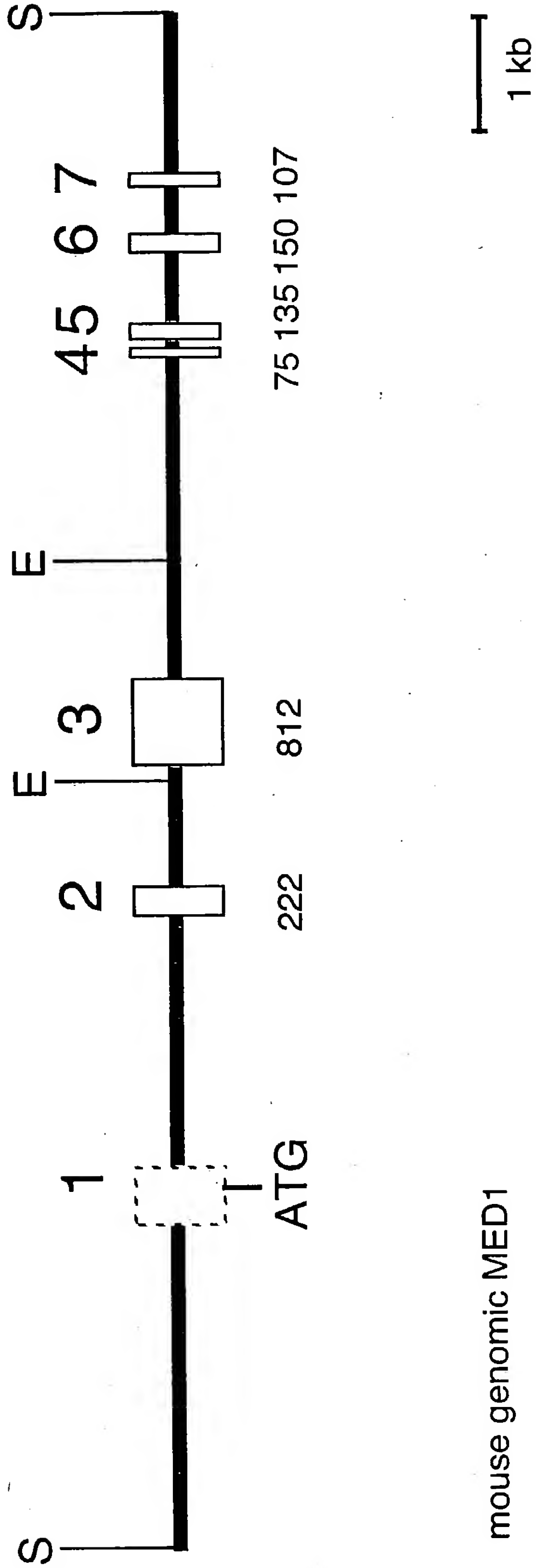


Fig. 14B



S
1
ATG
2
222
E
3
812
E
4 5
75 135
6
150
7
107
S
1 kb
mouse genomic MED1

Fig. 15

1 CAAGGAAGAT ATTGCTGTTG GACTGGGAGG AGTGGGAGAA GATGGAAAGG
51 ACCTGGTGAT AAGCAGTGAG CGCAGCTCCC TTCTCCAAGA GCCCACTGCT
101 TCTACTCTGT CTAGTACTAC AGCGACAGAA GGCCACAAGC CTGTCCCGTG
151 TGGATGGGAA AGAGTTGTGA AGCAAAGGTT ATCTGGGAAA ACTGCAGGAA
201 AATTTGATGT ATACTTTATC AGCCCACAAG GATTGAAGTT CAGATCAAAA
251 CGTTCACTTG CTAATTATCT TCTCAAAAAT GGGGAGACTT TTCTTAAGCC
301 TGAAGATTTT AATTTTACTG TACTGCCGAA AGGGAGCATC AATCCCGGTT
351 ATAAACACCA AAGTTTGGCA GCTCTGACTT CCCTGCAGCC AAATGAAACT
401 GACGTTTCAA AGCAGAACCT CAAGACACGA AGCAAGTGGA AAACAGATGT
451 GTTGCCTCTG CCCAGTGGTA CTTCAGAGTC GCCAGAAAGC AGCGGACTGT
501 CTAACTCTAA CTCGGCTTGC TTGCTATTGA GAGAACATAG GGACATTCAG
551 GATGTTGACT CTGAGAAGAG GAGAAAGTCC AAAAGAAAGG TGACTGTTTT
601 GAAAGGAACT GCAAGTCAGA AAACCAAACA AAAGTGCAGG AAGAGTCTCT
651 TAGAGTCTAC TCAAAGAAAC AGAAAAAGAG CATCTGTGGT TCAGAAGGTG
701 GGTGCTGATC GCGAGCTGGT GCCACAGGAA AGTCAACTCA ACAGAACCCT
751 CTGCCCTGCA GATGCCTGTG CAAGGGAGAC TGTTGGCCTG GCTGGGGAAG
801 AAAAATCACC AAGCCCAGGA CTGGATCTTT GTTTCATACA AGTAACTTCT
851 GGCACCACAA ACAAATTCCA TTCAACTGAA GCAGCAGGTG AAGCAAATCG
901 TGAGCAGACT TTTTTAGAAT CAGAGGAAAT CAGATCGAAG GGAGACAGAA
951 AGGGGGAGGC ACATTTGCAT ACTGGTGTTT TACAGGATGG CTCTGAAATG
1001 CCCAGCTGCT CACAAGCCAA GAAACACTTT ACTTCTGAGA CATTTCAAGA
1051 AGACAGCATC CCACGGACAC AAGTAGAAAA AAGGAAAACA AGCCTGTATT
1101 TTTCCAGCAA GTACAACAAA GAAGCTCTTA GCCCCCCAAG ACGCAAATCC
1151 TTCAAGAAAT GGACCCCTCC TCGGTCACCT TTTAATCTTG TTCAAGAAAT
1201 ACTTTTCCAT GACCCATGGA AGCTCCTCAT CGCGACTATA TTTCTCAATC
1251 GGACCTCAGG CAAGATGGCC ATCCCTGTGC TGTGGGAGTT TCTAGAGAAG
1301 TACCCTTCAG CTGAAGTGGC CCGAGCTGCC GACTGGAGGG ACGTGTCGGA

Fig. 16A

1351 GCTTCTCAAG CCTCTTGGTC TCTACGATCT CCGTGCAAAA ACCATTATCA

1401 AGTTCTCAGA TGAATATCTG ACAAAGCAGT GGAGGTATCC GATTGAGCTT

1451 CATGGGATTT GGTTAAAATA TGGCAACGAC TCTACCGGAT CTTTTGTGTC

1501 AATGAATGGA ACAG

Fig. 16B

mouse MED1 protein (upper sequence) x human MED1 protein (lower sequence)

```
  1 KEDIAVGLGGVGEDGKDLVI..SSERSSLLQEPTAST.LSSTTATEGHKP 47
    |||:|. |  ||||  .:.|  ||| . ||||| ||    .|  ||  |
 36 KEDVAMELERVGEDEEQMMIKRSSECNPLLQEPIASAQFGATAGTECRKS 85

 48 VPCGWERVVKQRLSGKTAGKFDVYFISPQGLKFRSKRSLANYLLKNGETF 97
    ||||||||||||| |||||:|||||||||||||||| |||||| |||||
 86 VPCGWERVVKQRLFGKTAGRFDVYFISPQGLKFRSKSSLANYLHKNGETS 135

 98 LKPEDFNFTVLPKGSINPGYKHQSLAALTSLQPNETDVSKQNLKTRSKWK 147
    ||||||.|||| |  |   ||  |:|||||   |:.. |  ||:|||| |
136 LKPEDFDFTVLSKRGIKSRYKDCSMAALTSHLQNQSNNSNWNLRTRSKCK 185

148 TDVLPLPSGTSESPESSGLSNSNSACLLLREHRDIQDVDSEKRRKSKRKV 197
     ||   ||..||  || ||||  |  |||:|   : ||.  | || | ||
186 KDVFMPPSSSSELQESRGLSNFTSTHLLLKEDEGVDDVNFRKVRKPKGKV 235

198 TVLKGTASQKTKQKCRKSLLESTQRNRKRAS................... 228
    |:|||   .|||. ||||     | . || |
236 TILKGIPIKKTKKGCRKSCSGFVQSDSKRESVCNKADAESEPVAQKSQLD 285

                          .
                          .
                          .
                          .

229 ...............EDSIPRTQVEKRKTSLYFSSKYNKEALSPPRRKSF 263
                   ||.|||||:|:||||||||||||||||||||||.|
386 CSPTRKDFTGEKIFQEDTIPRTQIERRKTSLYFSSKYNKEALSPPRRKAF 435

264 KKWTPPRSPFNLVQEILFHDPWKLLIATIFLNRTSGKMAIPVLWEFLELY 313
    ||||||||||||||| ||||||||||||||||||||||||||||.||| |
436 KKWTPPRSPFNLVQETLFHDPWKLLIATIFLNRTSGKMAIPVLWKFLEKY 485

314 PSAEVARAADWRDVSELLKPLGLYDLRAKTIIKFSDEYLTKQWRYPIELH 363
    ||||||| |||||||||||||||||||||||:|||||||||||:||||||
486 PSAEVARTADWRDVSELLKPLGLYDLRAKTIVKFSDEYLTKQWKYPIELH 535

364 GIWLKYGNDSYRIFCVNEWKQ 384
    ||  |||||||||||||||||
536 GIG.KYGNDSYRIFCVNEWKQ 555
```

Fig. 17

Fig. 18A

Exon 2

ggttttgtttttcc**ag**CAAGGAAGATATTGCTGTTGGACTGGGAGGAGTG
GGAGAAGATGGAAAGGACCTGGTGATAAGCAGTGAGCGCAGCTCCCTTCT
CCAAGAGCCCACTGCTTCTACTCTGTCTAGTACTACAGCGACAGAAGGCC
ACAAGCCTGTCCCGTGTGGATGGGAAAGAGTTGTGAAGCAAAGGTTATCT
GGGAAAACTGCAGGAAAATTTGATGTATACTTTATCAG**gt**aagcatttag
Gaaggaaaata

Fig. 18B

Exon 3

cttttttttttcctttta**ag**CCCACAAGGATTGAAGTTCAGATCAAAAC
GTTCACTTGCTAATTATCTTCTCAAAAATGGGGAGACTTTTCTTAAGCCT
GAAGATTTTAATTTTACTGTACTGCCGAAAGGGAGCATCAATCCCGGTTA
TAAACACCAAAGTTTGGCAGCTCTGACTTCCCTGCAGCCAAATGAAACTG
ACGTTTCAAAGCAGAACCTCAAGACACGAAGCAAGTGGAAAACAGATGTG
TTGCCTCTGCCCAGTGGTACTTCAGAGTCGCCAGAAAGCAGCGGACTGTC
TAACTCTAACTCGGCTTGCTTGCTATTGAGAGAACATAGGGACATTCAGG
ATGTTGACTCTGAGAAGAGGAGAAAGTCCAAAAGAAAGGTGACTGTTTTG
AAAGGAACTGCAAGTCAGAAAACCAAACAAAAGTGCAGGAAGAGTCTCTT
AGAGTCTACTCAAAGAAACAGAAAAAGAGCATCTGTGGTTCAGAAGGTGG
GTGCTGATCGCGAGCTGGTGCCACAGGAAAGTCAACTCAACAGAACCCTC
TGCCCTGCAGATGCCTGTGCAAGGGAGACTGTTGGCCTGGCTGGGGAAGA
AAAATCACCAAGCCCAGGACTGGATCTTTGTTTCATACAAGTAACTTCTG
GCACCACAAACAAATTCCATTCAACTGAAGCAGCAGGTGAAGCAAATCGT
GAGCAGACTTTTTTAGAATCAGAGGAAATCAGATCGAAGGGAGACAGAAA
GGGGGAGGCACATTTGCATACTGGTGTTTTACAGGATGGCTCTGAAATGC
CCAGCTGCTCACAAGCCAAGAAACACTTTACTTCTGAGACATTTCAAG**gt**
actcagtgcatgaaaa

Fig. 18C

Exon 4

gactataaactaattttgcttctc**ag**AAGACAGCATCCCACGGACACAAG
TAGAAAAAAGGAAAACAAGCCTGTATTTTTCCAGCAAGTACAACAAAGAA
G**gt**acccacctttccctaagc

Fig. 18D

Exon 5

tatatttntgn**ag**CTCTTAGCCCCCCAAGACGCAAATCCTTCAAGAAATG
GACCCCTCCTCGGTCACCTTTTAATCTTGTTCAAGAAATACTTTTCCATG
ACCCATGGAAGCTCCTCATCGCGACTATATTTCTCAATCGGACCTCAG**g**
ttnggggtcattgncat

Fig. 18E

Exon 6

tgtttatgctcccc**ag**GCAAGATGGCCATCCCTGTGCTGTGGGAGTTTCT
AGAGAAGTACCCTTCAGCTGAAGTGGCCCGAGCTGCCGACTGGAGGGACG
TGTCGGAGCTTCTCAAGCCTCTTGGTCTCTACGATCTCCGTGCAAAAACC
ATTATCAAGTTCTCAG**gt**atgtccccagcccag

Fig. 18F

Exon 7

tggatgtgtatccctc**ag**ATGAATATCTGACAAAGCAGTGGAGGTATCCG
ATTGAGCTTCATGGGATTTGGTTAAAATATGGCAACGACTCTACCGGAT
CTTTTGTGTCAATGAATGGAACAG**gt**aagcccaccactggggcc

Fig. 19A

Exon 1

GCGGCGGCGTCTGGGGCGCTTTCGCAACATTCAGACCTCGGTTGCAGCCCGGTGCCGTGAGCTGAA
GAGGTTTCACATCTTACTCCGCCCCACACCCTGGGCGTTGCGGCGCTGGGCTCGTTGCTGCAGCCG
GACCCTGCTCGATGGGCACGACTGGGCTGGAGAGTCTGAGTCTGGGGACCGCGGAGCTGCCCCCA
CCGTCACCTCTAGTGAGCGCCTAGTCCCAGACCCGCCGAATGACCTCCG**gt**aagttactgtcccct
tttgggcctcagtttcaccacctgtaaaatggtatcgggagagtggacagtgtgtgggcctttcta
acctttgacagagggtcggcanaaacctcgaagcccacgggtttagttactagggtctggagccca
ggtgctcttcctgtgcgatcagc...

Fig. 19B

Exon 2

...tttggaagacaggaaat**<u>actcccatagcacaagactgg</u>**tccacactgactttaatctccc
tcattttaatatggataatctatgtggttcctgcattgtcatggattaaaactgagtaggcagtgg
aagataaattttaaataagttaatcacttagactttgtttttcc**ag**CAAAGAAGATGTTGCTATGG
AATTGGAAAGAGTGGGAGAAGATGAGGAACAAATGATGATAAAAAGAAGCAGTGAATGTAATCCCT
TGCTACAAGAACCCATCGCTTCTGCTCAGTTTGGTGCTACTGCAGGAACAGAATGCCGTAAGTCTG
TCCCATGTGGATGGGAAAGAGTTGTGAAGCAAAGGTTATTTGGGAAGACAGCAGGAAGATTTGATG
TGTACTTTATCAG**gt**aagcatataagatggtaaagatagtacagccaaatgattttgtctgg**<u>gcag</u>**
<u>gtagtgggagcatagc</u>aggaatcttagcttctttatatttttaccataaaaccattgcagattc
tattctttcaatgttgctattaattacatcaagtgatttggggaaaattacatacattttgtccct
ccttctgtgaatggttaacgggtaggttgcattttagttatatttataaatttatattgtcataga
ggaaccatttaaaaggccattatcactctttttcatttttaaatgacagagacctatggcaacatt
tggaaattaattagaatctgaaatgtggtccagttcttttaaaagtcccttctatttactagcagt
aagtttcctttaatatcattttct**ag(continues into exon 3, see below)**

Fig. 19C

Exon 3

aatctgaaatgtggtccagttcttttaaaagt**ccctt****ctatttactagcagta**agtttccttt
aatatcattttct**ag**CCCACAAGGACTGAAGTTCAGATCCAAAAGTTCACTTGCTAATTATCTTCA
CAAAAATGGAGAGACTTCTCTTAAGCCAGAAGATTTTGATTTTACTGTACTTTCTAAAAGGGGTAT
CAAGTCAAGATATAAAGACTGCAGCATGGCAGCCCTGACATCCCATCTACAAAACCAAAGTAACAA
TTCAAACTGGAACCTCAGGACCCGAAGCAAGTGCAAAAAGGATGTGTTTATGCCGCCAAGTAGTAG
TTCAGAGTTGCAGGAGAGCAGAGGACTCTCTAACTTTACTTCCACTCATTTGCTTTTGAAAGAAGA
TGAGGGTGTTGATGATGTTAACTTCAGAAAGGTTAGAAAGCCCAAAGGAAAGGTGACTATTTTGAA
AGGAATCCCAATTAAGAAAACTAAAAAAGGATGTAGGAAGAGCTGTTCAGGTTTTGTTCAAAGTGA
TAGCAAAAGANAATCTGTGTGTAATAAAGCAGATGCTGAAAGTGAACCTGTTGCACAAAAAAGTCA
GCTTGATAGAACTGTCTGCATTTCTGATGCTGGAGCATGTGGTGAGACCCTCAGTGTGAGCAGTGA
AGAAAACNGCCTTGTAAAAAAAAAAGAAAGATCATTGAGTTCAGGATCAAATTTTTGTTCTGAACA
AAAAACTTCTGGCATCATAAACAAATTTTGTTCAGCCAAAGACTCAGAACACAACGAGAAGTATGA
GGATACCTTTTTAGAATCTGAAGAAATCGGAACAAAAGTAGAAGTTGTGGAAAGGAAAGAACATTT
GCATACTGACATTTTAAAACGTGGCTCTGAAATGGACAACAACTGCTCACCAACCAGGAAAGACTT
CACTG**gt**gagaaaatatttcaag**gt**atccagtgctttcagcactattaaacattagtgat**gagaa**
atttatatgctgcatctgtatcgtgccatac

Please note: at the end of exon 3, two alternative splice donor sites are present (see Sequence Variations, page 40 of the application).

Fig. 19D

Exon 4 and Exon 5

tagtaccaagttcatgggtcattagttagattaattgggtatttatgtaaagggcttagaatagtg
cctggcatgctttgtaatagtgttgatattattatt**tgcatccctcaatattgcttt**aagcta
aaccatagactccataaagtgtttacttttccttttc**ag**AAGATACCATCCCACGAACACAGATAG
AAAGAAGGAAAACAAGCCTGTATTTTTCCAGCAAATATAACAAAGAAG**gt**atccctttcccaatca
gaacagcaaattctaattccattttgggttttcaattctgatgcactatgtttgttt**ag**CTCTTAG
CCCCCCACGACGTAAAGCCTTTAAGAAATGGACACCTCCTCGGTCACCTTTTAATCTCGTTCAAGA
AACACTTTTTCATGATCCATGGAAGCTTCTCATCGCTACTATATTTCTCAATCGGACCTCAG**gt**tt
ggggattattatcatctttgtcttagtagagacagtgtggt**agggagaaagcactgaattga**g
gcctgggttcaaagtcattttgagtgtgtcacctgggatagggcattccccctttcacccttaaac
tcttcacctatgaggaaaatggggg

Fig. 19E

Exon 6

ccagtgtttttgtttttgttttctttaaaaaaaaaaaaaaccctctggatgagatttctatga
gaaactacttgaacgtgaaatcagcccacctggagtcttgtaatcattcagttacttttacnttcc
c**ag**GCAAAATGGCAATACCTGTGCTTTGGAAGTTTCTGGAGAAGTATCCTTCAGCTGAGGTAGCAA
GAACCGCAGACTGGAGAGATGTGTCAGAACTTCTTAAACCTCTTGGTCTCTACGATCTTCGGGCAA
AAACCATTGTCAAGTTCTCAG**gt**attttcctatacacccaaaggaaaaacataatacattgtgctt
atttaa**gagagccacaccttaaacttt**aatgttctcagatactatattaatggaggtttttca
gctcaagcatttaaaaaagtccacttttccccaaaccacagtctcccactgacctaaacaataaat
cttt

Fig. 19F

Exon 7

cttta**gaagctgacctgataatgtgg**gatgttgtattcttc**ag**ATGAATACCTGACAAAGCAG
TGGAAGTATCCAATTGAGCTTCATGGGATTGGTAAATATGGCAACGACTCTTACCGAATTTTTTGT
GTCAATGAGTGGAAGCAG**gt**gaggctcactcccatccataattcagcacatt**tggtctctgagg**
caaaataagtccaccattatggttaagacnatttattggggatacaaatgctattacagtcacaa
caattgtgttcctggctgcggggaagcgngtggcatgtgggttttggggttttgatcagtaggcg
ctcccagg

Fig. 19G

Exon 8

tgtgtgagattaccttaatataaggtataacttaaaatattcatgaatcccaggaggttaaaggtt
ataacttttaggtatgg**tatcgtaatgtactgtccccc**agcaaacatttaaaaagccaatttt
aaaaaatgtatttctgactaagttacatntaaggtctctgcctctgtatcttatgtttcttcc**ag**G
TGCACCCTGAAGACCACAAATTAAATAAATATCATGACTGGCTTTCCCAAAATCATGAAAAATTAA
GTTTATCTTAAACTCTGCAGCTTTCAAGCTCATCTGTTATGCATAGCTTTGCACTTCAAAAAAGCT
TAATTAAGTACAACCAACCACCTTTCCAGCCATAGAGATTTTAATTAGCCCAACTAGAAGCCTAGT
GTGTGTGCTTTCTTAATGTGTGTGCCAATGGTGGATCTTTGCTACTGAATGTGTTTGAACATGTTT
TGAGATTTTTTTAAAATAAATTATTATTTGACAACA*atccaaaaaaaatacggcttttccaatga
tgaaatataatcagaagatgaaaaatagttctaaactatcaataatacaaagcaaat**ttctatca**
gccttgctaaagctaggggcccactaaatatttt

Please note: asterisk indicates the poly(A) addition site.

Fig. 19H

Complete sequence of the intron between exon 7 and exon 8.

GGAAGCAGgtgaggctcactcccatccataattcagcacatttggtctctgaggcaaaataagtcc
accattatggttaagactatttattggatacaaatgctattacagtcacaaacaattgtgttcctg
gctgcggggaagcgagtggcatgtgggttttggggttttgatcagtaagcgctcccaagtccaca
aagaccagtccagcggcgtggcctctgactcatctccagtggtttgtcacctctggccctgttcct
gtcattccctatttgtgtgctatctctaagcctgacgtggttttcctcctgtcaaaagtacaccac
tacaggaaagcaggaaggtttgggccttgcaatgtatgcatattgggtttctcttagtggtctcag
actacgtttgtggtgactgggtcctgcttcagccctgttgaatatgcccagcctgtggcatgctgg
tggtcatcctggcagctggtgggtggcctggtatgctgcccactcagcttgagactcaccctcatg
cattcagccagtaggtctggccaagcctgaactgaaggaccatggtcctatcccagcttcatcaca
gcaatccattgtgacctgagaatccatttaacctctcggtctagaacctccttctggaaagtgagg
tattaatacttgactcaatgttatcgccaccccacattctaagtcatggttgagtagtaatttgga
cagtaccttgtaaattgtgtgagattaccttaatataaggtataacttaaaatattcatgaatccc
aggaggttaaaggttataacttttaggtatggtatcgtaatgtactgtcccccagcaaacatttaa
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tttcttccagGTGCACCC

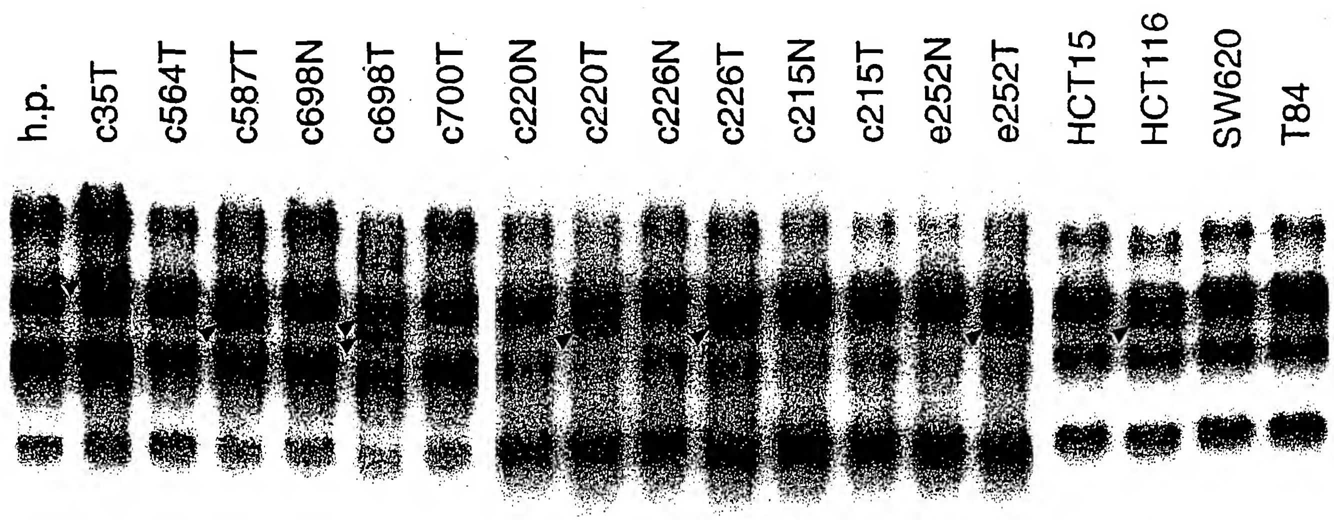

h.p.
c35T
c564T
c587T
c698N
c698T
c700T
c220N
c220T
c226N
c226T
c215N
c215T
e252N
e252T
HCT15
HCT116
SW620
T84

Fig. 20A

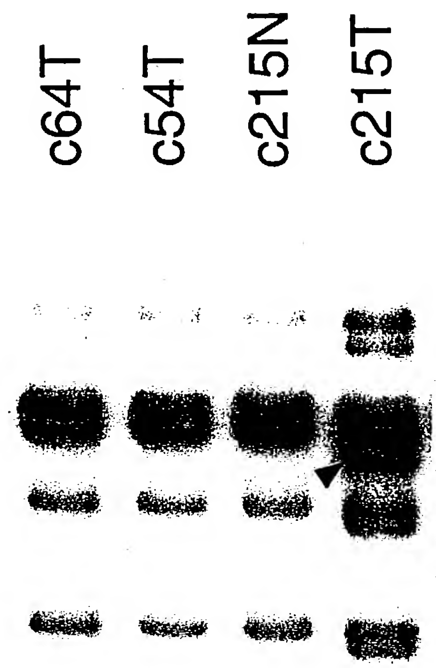


Fig. 20B

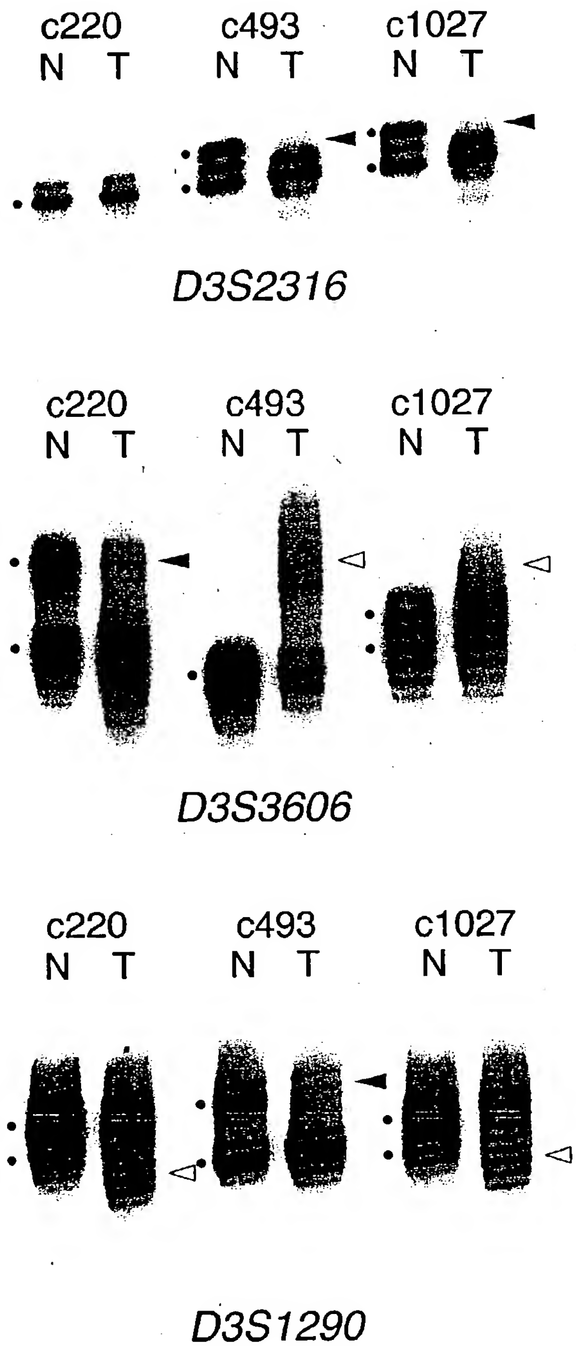

c220
N T
c493
N T
c1027
N T
D3S2316
c220
N T
c493
N T
c1027
N T
D3S3606
c220
N T
c493
N T
c1027
N T
D3S1290

Fig. 20C

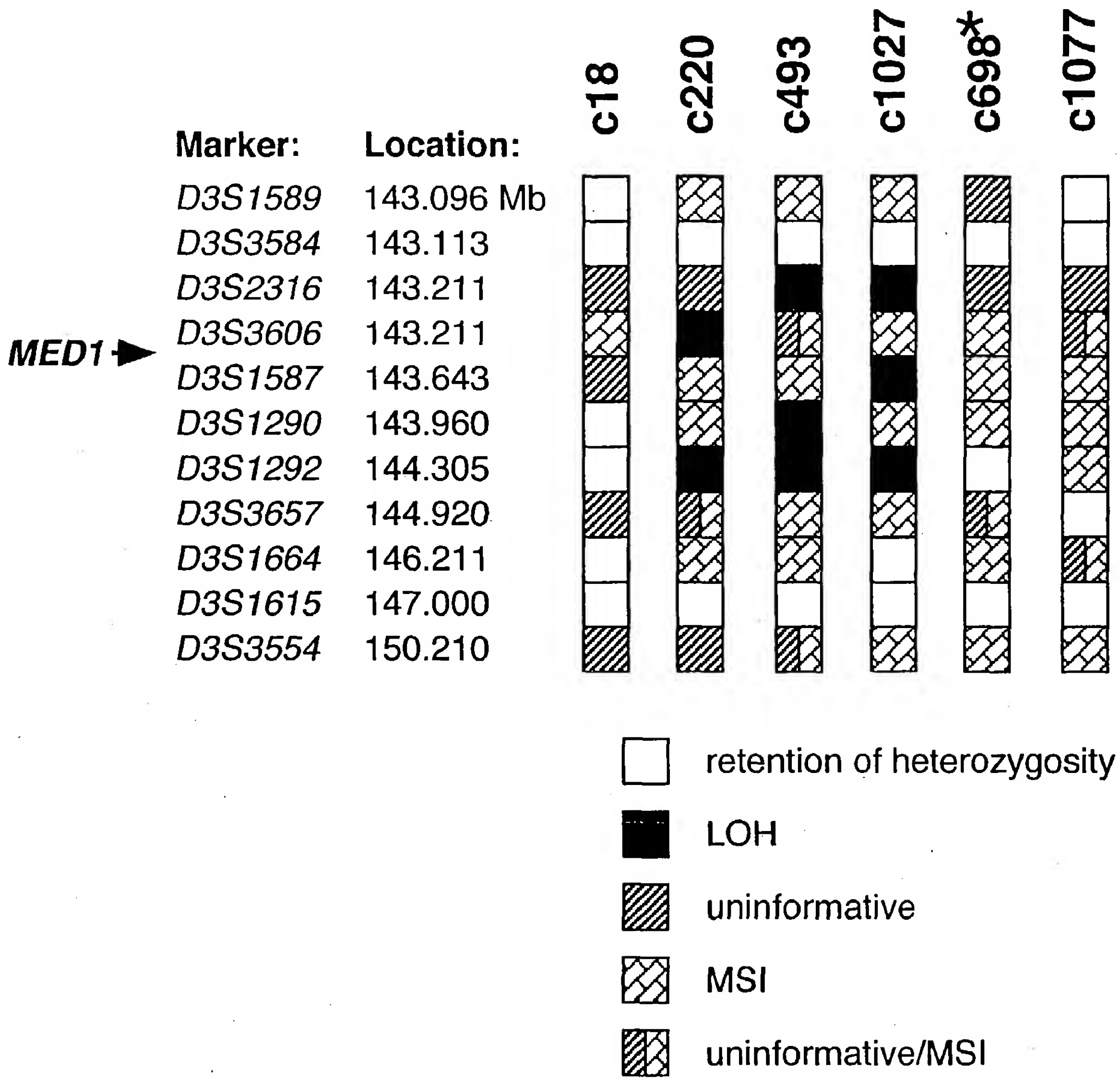

c18
c220
c493
c1027
c698*
c1077
Marker:
Location:
D3S1589
143.096 Mb
D3S3584
143.113
D3S2316
143.211
MED1
D3S3606
143.211
D3S1587
143.643
D3S1290
143.960
D3S1292
144.305
D3S3657
144.920
D3S1664
146.211
D3S1615
147.000
D3S3554
150.210
retention of heterozygosity
LOH
uninformative
MSI
uninformative/MSI

Fig. 20D

MED1 glycosylase assay

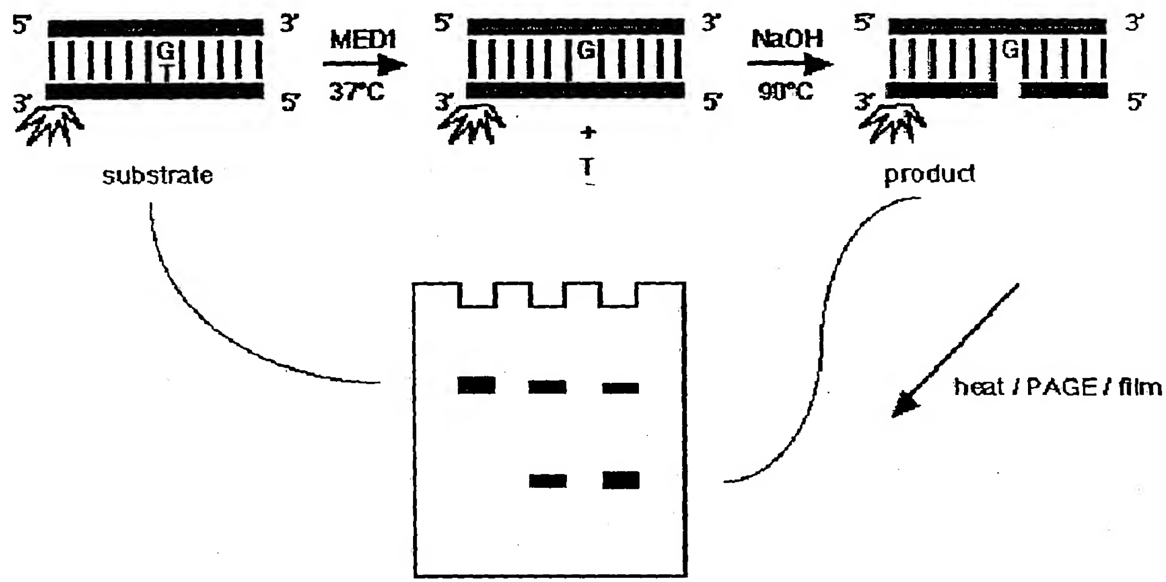
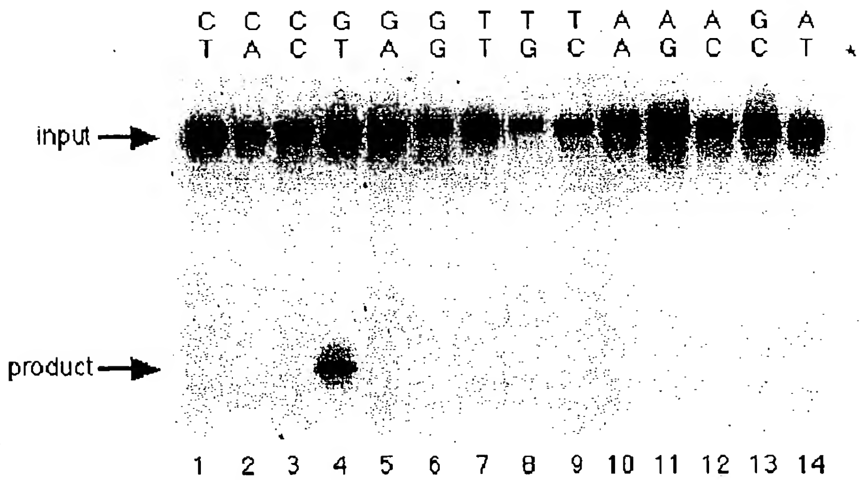


Fig. 21



* The asterisk indicates that the bottom oligonucleotide strand is radioactively labelled

Fig. 22

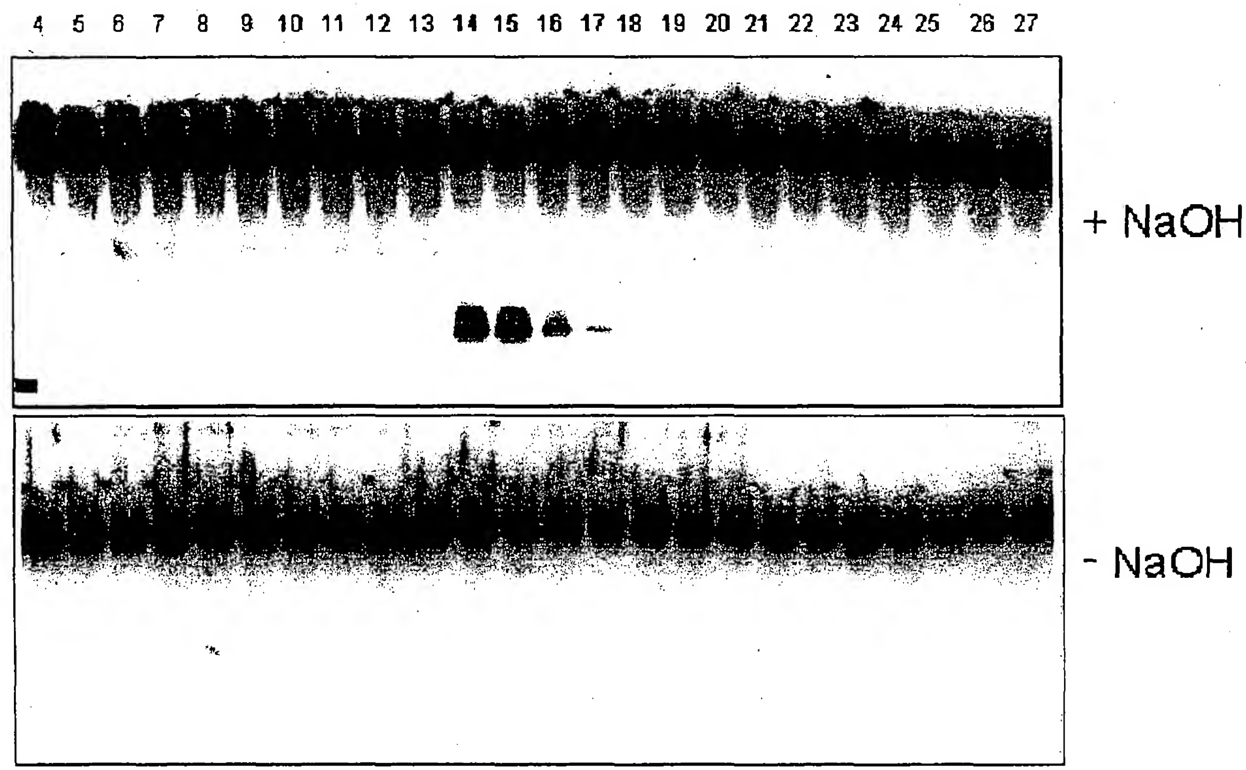

4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
+ NaOH
- NaOH

Fig. 23

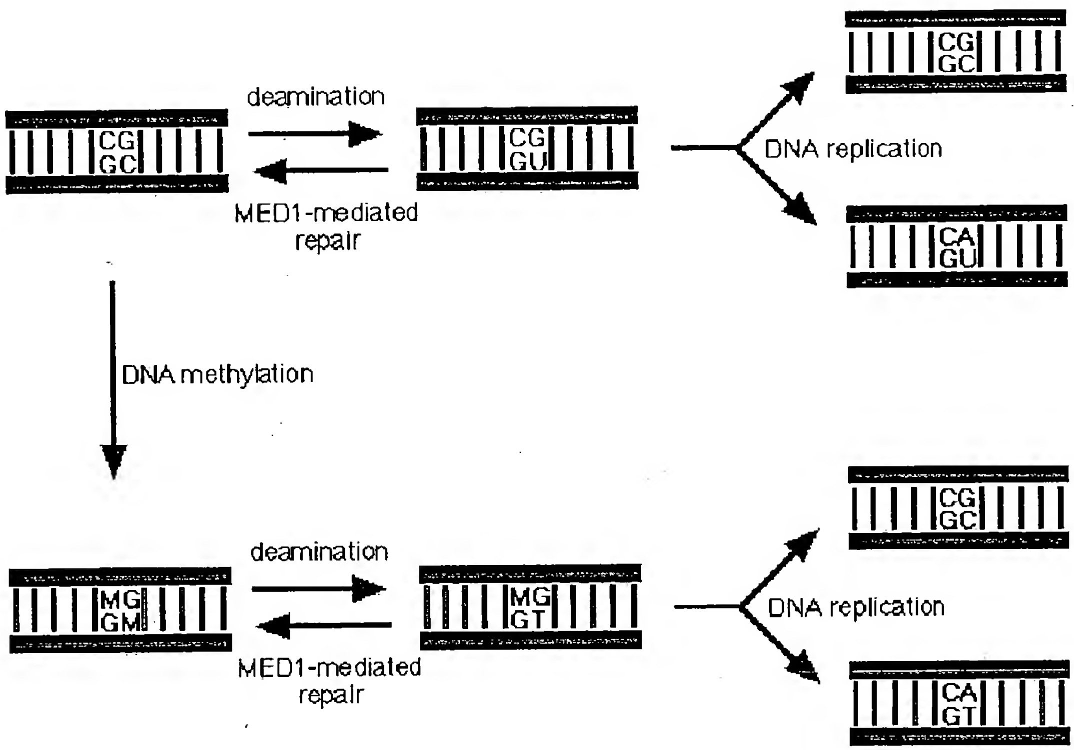

deamination
CG
GC
CG
GU
MED1-mediated repair
DNA replication
CG
GC
CA
GU
DNA methylation
deamination
MG
GM
MG
GT
MED1-mediated repair
DNA replication
CG
GC
CA
GT

Fig. 24

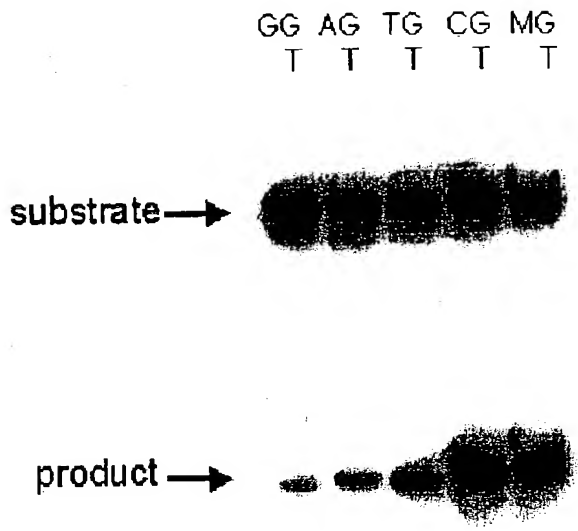


Fig. 25

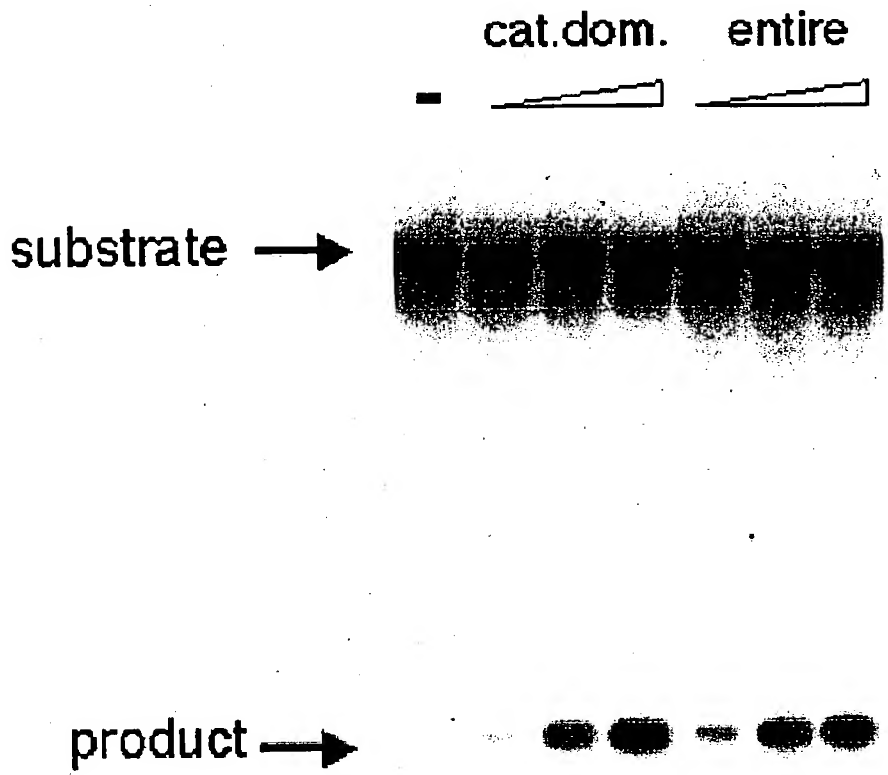
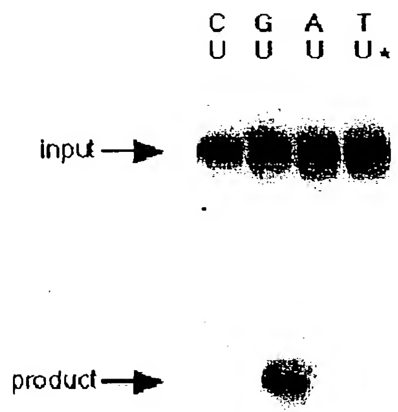


Fig. 26



*The asterisk indicates that the bottom oligonucleotide strand is radioactively labelled

Fig. 27

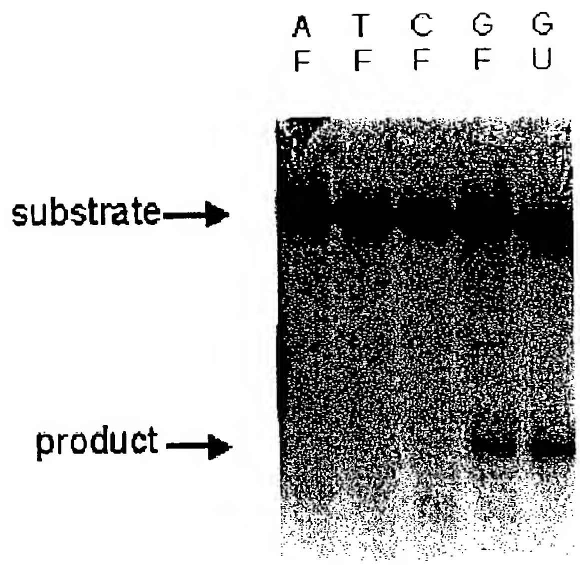
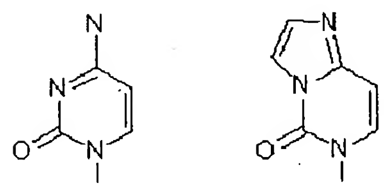


Fig. 28



Cytosine **3, N^4-Etheno-cytosine (E)**

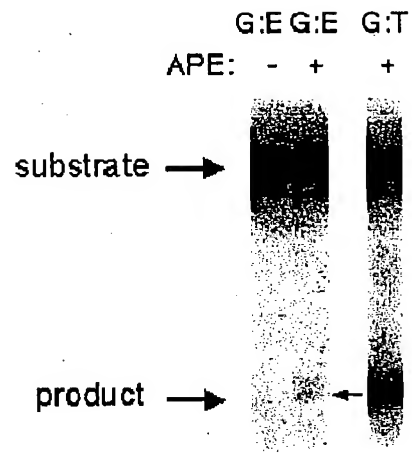


Fig. 29

1) Denature DNA fragments and mix with CT-SNP probe in order to generate heteroduplex

5′ ____CpG____3′ → 5′ ____CpG____3′ heteroduplex
3′ ____GpC____5′ → 3′ ____GpT____5′ with G:T mismatch

allele 1

or

5′ ____CpA____3′ → 5′ ____CpA____3′ homoduplex
3′ ____GpT____5′ → 3′ ____GpT____5′

allele 2

+

3′ ____GpT____5′

CT-SNP probe

2) Incubate annealed molecules with recombinant MED1 followed by NaOH in order to cleave heteroduplex

+ MED1
+ NaOH

5′ ____CpG____3′ or 5′ ____CpA____3′
3′ ____Gp ____5′ or 3′ ____GpT____5′

cleaved heteroduplex uncleaved homoduplex

3) Separate fragments of the cleaved strand by standard techniques (e.g. by electrophoresis)

Fig. 30